Effects of Low Concentrations of Benzene on Mouse Hematopoietic Cells *in Vivo*: A Preliminary Report

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Evaluation of benzene-induced hematotoxicity following exposure to low concentrations is important for understanding mechanisms of toxicity and determining the dose response at benzene levels close to the current occupational exposure limit (1 ppm). Male B6C3F1 mice were exposed to 0, 1, 10, 100, or 200 ppm benzene by inhalation for 6 hr/day, 5 days/week for 1, 2, 4, or 8 weeks. At each sampling time, we evaluated primitive and committed progenitor cells, differentiating and maturing lineage-specific cells, and stromal cells in the bone marrow; T and B lymphocytes of the spleen and thymus; micronucleated reticulocytes and erythrocytes; and standard blood parameters. At 100 and 200 ppm benzene, there were rapid and significant reductions in number of reticulocytes in the blood, B lymphocytes in the bone marrow and spleen, and an increased frequency of micronucleated reticulocytes in the bone marrow. At 10 ppm, the only parameter affected was a transient reduction in the number of splenic B lymphocytes. There were no significant effects induced by 1 ppm benzene in this study. The present study suggests numbers of B lymphocytes and maturing erythrocytes, and frequency of micronucleated reticulocytes are sensitive indicators of benzene-induced hematotoxicity and will be useful in further investigation of the hematotoxicity induced by 10 to 100 ppm benzene. — Environ Health Perspect 104(Suppl 6):1275–1276 (1996)

Key words: benzene, hematotoxicity, micronuclei, mouse, inhalation

Introduction

Benzene-induced hematotoxicity is poorly understood. Several hypotheses regarding potential mechanisms of benzene hematotoxicity have been based on results of *in vivo* experiments that investigated only one or two parameters of hematopoiesis. Most proposed mechanisms of benzene-induced effects such as cytotoxicity, apoptosis, mutagenesis, and cell replication can be demonstrated *in vitro* at relatively high concentrations of benzene metabolites (*1,2*). Benzene effects *in vitro* do not necessarily correlate with benzene-induced hematotoxicity following *in vivo* exposures to low concentrations, since *in vitro* assays of hematopoiesis lack the complex hematopoietic cell interrelationships, fine-tuned regulation, and compensation mechanisms that are essential in normal hematopoiesis.

The association of severe bone marrow damage, as manifested by aplastic anemia, with the progression to acute myeloid leukemia (AML) that has occurred in some humans exposed to high concentrations of benzene (*3,4*) is also poorly understood. If a regenerative increase in replication of stem cells and progenitor cells plays a primary role in benzene-induced leukemia, then the risk of developing AML might be predicted from the association of benzene-induced cytotoxicity with regeneration and simultaneous mutagenesis.

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Abbreviation used: AML, acute myeloid leukemia; ANOVA, analysis of variance; BrdU, bromodeoxyuridine; CFU-E, colony-forming units–erythrocytic; CFU-GM colony-forming units–granulocytic and macrophage; CFU-HPP, colony-forming units–high proliferative potential cells.

The present study has three purposes: it investigates benzene-induced hematotoxicity induced by low-concentration exposure to benzene *in vivo*; it presents a model of the cell populations adversely affected by benzene and the progression of this damage; and it investigates the ability of the hematopoietic tissues of the mice to compensate or adapt with continued exposure. The frequency of micronucleated reticulocytes and erythrocytes during benzene exposure was measured as an indicator of mutagenesis.

The most important aspects of benzene-induced hematotoxicity are the possible toxic effects caused by low concentrations of benzene (1 and 10 ppm). Concurrent assessment of toxicity elicited by high concentrations of benzene (100 and 200 ppm) established a reference for comparison. This communication provides preliminary information about the effects of benzene inhalation by mice at low concentrations on hematopoiesis, lymphopoiesis, and mutagenicity.

Methods

Male B6C3F1 12-week-old mice inhaled 0, 1, 10, 100, or 200 ppm benzene for 6 hr/day, 5 days/week for 1, 2, 4, or 8 weeks. Animals were removed from the inhalation chambers at each sampling time following the last scheduled exposure and were immediately evaluated for hematotoxicity and mutagenicity. Several stages of hematopoietic cell replication, differentiation, and maturation starting at a very primitive progenitor-cell compartment in the bone marrow through to the mature cells in the blood were examined. Bone marrow from individual mice was evaluated for number and replication of high proliferative-potential cells (CFU-HPP) using a previously described culture assay (*5*). The number of granulocyte and macrophage (CFU-GM), as well as erythroid cells (CFU-E) were evaluated using agar culture (*6*) and a complete methylcellulose medium (StemCell Technologies, Vancouver, BC), respectively. The number and replication of erythropoietic cells, granulopoietic cells, and B lymphocytes in the bone marrow were evaluated using monoclonal antibodies to surface antigens and BrdU incorporation. Stromal cells in the bone marrow were evaluated by counting adherent cells after 24 hr in culture. Conventional hematology parameters were recorded including reticulocyte counts. The frequency of micronucleated

reticulocytes and erythrocytes in the bone marrow and blood were analyzed by flow cytometry and manual counts (*7*).

For each sampling time, the data from each benzene exposure group were compared to the controls using Dunnett's one-way analysis of variance (ANOVA).

Results

Inhalation of 1 ppm benzene extending over an 8-week period did not cause statistically significant changes in any of the parameters assessed compared to controls.

At 10 ppm, benzene also caused a transient decrease in the number of splenic B lymphocytes at 2 weeks with a return to control numbers at 4 weeks.

Exposure of mice to 100 and 200 ppm benzene for 5 days caused a reduction in the number of differentiating and maturing cells of the three major lineages (B lymphocytes, granulocytes, erythrocytes) in the bone marrow, splenic lymphocytes, blood reticulocytes and erythrocytes, and blood leukocytes. The number of maturing B lymphocytes decreased progressively in the bone marrow of mice exposed to 200 ppm benzene over the 8 weeks of exposure (Figure 1). An increase in the number of B lymphocytic cells in the mice exposed to

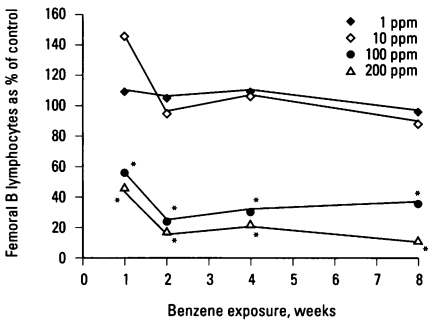

Figure 1. Effects of benzene on the absolute number of B lymphocytic cells in the bone marrow (pro-B lymphocytes through mature B lymphocytes), presented as a percentage of the control values (mice exposed to air) for each time point. Asterisk indicates a statistically significant reduction compared to controls, $p < 0.05$.

10 ppm for 5 days, as compared to controls, was due to the number of total bone marrow cells. The total number of bone marrow cells was within the range of control male B6C3F1 mice and this increase, compared to controls, was not reproducible in subsequent studies. Within 2 weeks the number of high proliferative-potential progenitor cells and committed progenitor cells (CFU–GM and CFU–E) decreased. The percentage of B-lymphocytic cells and CFU–HPP cells in S phase increased. The blood reticulocytes decreased rapidly in mice exposed to 200 ppm benzene and over the following 7 weeks of exposure the numbers increased (Figure 2). Exposure to 100 and 200 ppm benzene also caused an increased frequency of micronucleated reticulocytes in the bone marrow and micronucleated erythrocytes in the blood after 5 days of exposure.

Discussion

Inhalation of 1 ppm benzene by male B6C3F1 mice over an 8-week period did not induce significant hematotoxicity in this study. No statistically significant change in the frequency of micronucleated reticulocytes or erythrocytes was induced by 1 or 10 ppm benzene. The concentration response of micronucleated reticulocytes and erythrocytes with 1 to 200 ppm benzene in mice from this study has been described (*7*). A quadratic concentration–response model fit to frequency of bone marrow micronucleated reticulocyte data of mice exposed to a maximum of 200 ppm benzene had a high correlation and could not be rejected based on lack of fit. A linear concentration–response model was rejected based on a lack of fit of the data.

The hematotoxic effects of 100 ppm benzene were not as severe as exposure to 200 ppm benzene, and there was a return toward control values at week 8 as compared to 2 and 4 weeks, indicating compensation by the bone marrow with continued exposure. Figure 1 demonstrates a mild improvement in the number of

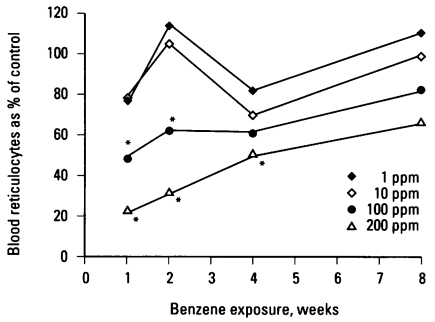

Figure 2. Effects of benzene on the number of reticulocytes per µl of blood, presented as a percentage of the control values (mice exposed to air) for each time point. Asterisk indicates a statistically significant reduction compared to controls, $p < 0.05$.

B lymphocytes in the bone marrow at 8 weeks of exposure to 100 ppm (38% of control) as compared to 2 weeks (26% of control). An increased replication of primitive progenitor cells (CFU–HPP) and B lymphocytes were two measured responses in this study that indicated an attempt by the bone marrow to increase progenitor-cell replication to compensate for the very early loss of maturing cells.

The present study suggests that decreased number of B lymphocytes and maturing erythrocytes, and increased frequency of micronucleated reticulocytes are sensitive indicators of benzene-induced hematotoxicity. The decreased number of blood reticulocytes was a useful parameter to measure in an acute exposure (1–5 days), whereas the decreased number of femoral or splenic B lymphocytes and increased frequency of femoral micronucleated reticulocytes were measurable effects for a longer period (up to 8 weeks). Two weeks of exposure was the optimal timepoint to assess benzene damage for most of the parameters evaluated in this study, because bone marrow compensation by increasing progenitor-cell replication and replenishing cell loss appears to be occurring during continued exposure to benzene.

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